OSMOTIC TOLERANCE AND REGULATION IN CRABS FROM A HYPERSALINE LAGOON

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It has been well established that the semi-terrestrial crab *Pachygrapsus crassipes* can osmo-regulate strongly in both dilute and concentrated sea water (Jones, 1941; Prosser *et al.*, 1955; Gross, 1955). On the other hand *Hemigrapsus oregonensis*, which is a less terrestrial crab than *Pachygrapsus*, was shown by Jones (1941) to be a strong hyper-osmotic regulator (maintenance of blood concentration above the concentration of the external medium), but was incapable of hypo-osmotic regulation in concentrated sea water after an immersion period of 72 hours. Gross (1957a), however, demonstrated hypo-osmotic regulation for *Hemigrapsus* in 150% sea water for a period of about 20 hours. The osmotic behavior of these two crabs fits well into the correlation between terrestrial behavior and hypo-osmotic regulation first indicated by Jones (1941) and expanded by Gross (1955). That is, crabs tending toward life on land are able to regulate in concentrated sea water and this in turn is accompanied by the additional ability to regulate in dilute sea water.

The adaptive significance of osmotic regulation in terrestrial and semi-terrestrial crabs was questioned by Gross (1955), inasmuch as adult land crabs will drown when completely immersed. Also, the semi-terrestrial *Pachygrapsus* is but rarely found in salinities deviating much from normal and, in fact, shows a precise preference for normal sea water. Thus, this highly active crab will avoid osmotic stress and is capable of seeking the normal salinities of the open sea (Gross, 1957b).

In January of 1959, Los Penasquitos lagoon, near San Diego, California, became isolated from the open sea by a sand bar. In early summer it was observed that large populations of *Pachygrapsus crassipes* and *Hemigrapsus oregonensis* and a relatively small population of the fiddler crab, *Uca crenulata*, were trapped but thriving in the lagoon where salinities were in excess of 150% sea water (5.3% salt). This was the first situation in which the author had found *Pachygrapsus* in the field where it was exposed to osmotic stress yet could not reach normal sea water. Although a complete field study of the environmental salinities of *Uca crenulata* has not been made, it is known to be a strong hypo-regulator (Jones, 1941) and is commonly known to live in back-bay areas where high salinities would be expected. On the other hand, the presence of *Hemigrapsus* in a hyper-saline lagoon was indeed surprising and suggested that contrary to laboratory findings was probably demonstrating hypo-regulation of significant magnitude and duration.

This investigation is concerned with ionic and osmotic regulation in *Hemigrapsus* and *Pachygrapsus* collected from Los Penasquitos lagoon during the period from July 1, 1959, to December 2, 1960, when the lagoon remained isolated from the open sea. Physical and other biological aspects of the lagoon during parts of this period are presented by Carpelan (1961).

MATERIALS AND METHODS

Blood and urine were sampled from the crabs as previously described (Gross, 1959). In the field blood and urine were taken from the animals immediately after capture. The samples were then placed into cold vials and quick-frozen on dry-ice. Also, sea water samples were taken at the site of capture and from different depths as a check for stratification. All samples were then returned to the laboratory for analysis. Salinity was determined by conductivity, using a 1000-cycle bridge. Na and K were determined by flame photometry; Ca and Mg by titration with ethylene diamine tetra acetic acid (EDTA) as previously described by Gross (1959). Melting points of blood and urine were made by the method of Gross (1954). Salinity preference was determined by means of a selectivity chamber (Gross, 1957b).

RESULTS

Figure 1 presents the blood concentrations of both *Pachygrapsus* and *Hemigrapsus* sampled in the field at various dates and salinities. Blood concentrations (% sea water) are represented by: (a) total cations (mM/l.) (Na, K, Ca and Mg) relative to those found in normal sea water, and (b) by melting points. For seventeen crabs on which both blood melting point and total cations were determined, the ratio, $\frac{\text{melting point (\% sea water)}}{\text{total cations (\% sea water)}} = 0.98$, S.D. = 0.006. Thus, total cations (mM/l.) can be converted with sufficient precision to melting point and either type of determination will show the degree of osmotic regulation demonstrated by the crabs. Although the salinities of the lagoon water differed from site to site, those indicated in Figure 1 represent the media from which the crabs were actually collected.

It thus can be seen that *Hemigrapsus* (indicated by circles) was indeed regulating osmotically in the hypersaline water. Regulation was particularly strong in 160% sea water, but some *Hemigrapsus* were maintaining the blood concentration considerably below the ambient medium of 175% sea water (August 25, 1959). When the lagoon water reached approximately 180% sea water in mid-September, 1959, hypo-osmotic regulation in *Hemigrapsus* had essentially broken down; only one crab could be considered to be regulating with any strength. It also should be noted that the blood of several crabs was above the concentration of the indicated medium. This perhaps can be explained by the fact that most crabs observed (both species) at this time were piled up at the interphase between air and water, having migrated out of the depths of the lagoon. It is, therefore, likely that crabs captured when immersed had been out of the water and exposed to evaporation which had in turn elevated the blood osmotic pressure. While there were many dead and moribund *Hemigrapsus* at the edges of the lagoon at this time, only active specimens were sampled.

When the lagoon water reached a concentration of about 190% sea water no living *Hemigrapsus* could be found, although *Pachygrapsus* (indicated by triangles) were still surviving and active in these salinities. It cannot be concluded from these data, however, that the upper limits of salinity in which *Hemigrapsus* can survive in nature lie somewhere between 180% and 190% sea water because there are many other physical and biological factors involved.

As was expected some *Pachygrapsus* were demonstrating strong hypo-regulation in all the salinities indicated in Figure 1.

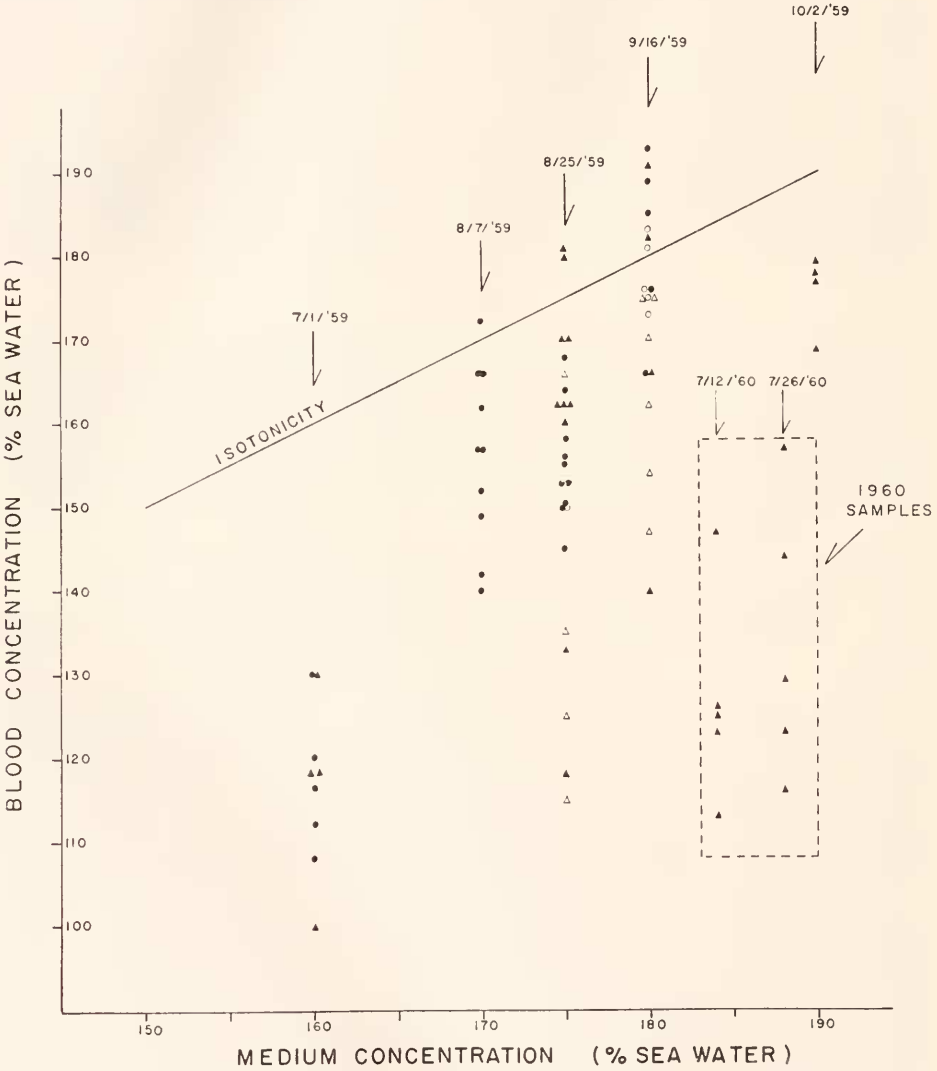

FIGURE 1. Osmotic regulation and tolerance by *Pachygrapsus* and *Hemigrapsus* in Los Penasquitos lagoon. Blood concentrations for individual crabs are represented by circles for *Hemigrapsus;* by triangles for *Pachygrapsus.* Each point represents a different individual. Blood concentrations by melting point determination are indicated by open points; blood concentrations by total cations (mM/l.) are indicated by solid points. Dates of sampling are indicated by arrows. The 1960 samples are enclosed by broken line.

An interesting phenomenon demonstrated by both species in Figure 1 is their ability to tolerate high blood concentrations. Thus, active *Hemigrapsus* and *Pachygrapsus* had blood concentrations which were close to isotonic or hypertonic to 180% sea water.

In December, 1959, the lagoon was filled by the high tides and the salinity dropped to 109% sea water although the isolating bar remained intact. However, no living *Pachygrapsus* or *Hemigrapsus* could be found. Nevertheless, in the summer of 1960, both *Pachygrapsus* and *Hemigrapsus* were found in relatively small numbers in the isolated lagoon which again had become hypersaline. The specimens of *Hemigrapsus* were sufficiently small to have washed over the bar during the winter high tides in the larval stage and developed in the lagoon. Most *Pachygrapsus* were mature and therefore either entered the lagoon across the bar in the adult stage from a sandy beach not likely to be inhabited by this species or were remnants from the 1959 population. Blood concentrations of the 1960 *Pachygrapsus* samples (enclosed by broken line in Figure 1) show that hypo-osmotic regulation was stronger in the 1960 crabs than in the 1959 crabs. The combined 1960 blood samples taken from animals immersed in salinities greater than 180% sea water concentrations were lower in concentration on the average than the 1959 samples taken from crabs immersed in 175% sea water, $P < 0.01$. This suggests that rigorous selection had taken place which favored hypo-regulation in *Pachygrapsus,* and that the 1960 specimens were survivors of this selection from the 1959 population. As indicated above, the salinity of the lagoon water in early 1960 was close to normal. Thus, the 1960 crabs were not regulating more strongly than those collected in 1959 because of long-term acclimatization, although two successive seasons of exposure to increasing salinities might have had the same effect.

While immature *Hemigrapsus* were captured from the lagoon in 1960 when the maximum salinity was 188% sea water, samples of sea water from the sites of capture were not taken. Thus, the degree of regulation for this species cannot be told for 1960. It is interesting, however, that two pooled samples of blood from these small specimens were approximately isotonic to the maximum salinity found in the lagoon at that time (188% sea water).

It is apparent from the data in Figure 1 that contrary to laboratory finding (Jones, 1941; Gross, 1957a) *Hemigrapsus* in nature is quite capable of strong hypo-osmotic regulation. The contradiction between laboratory studies and the field studies of the present investigation could be caused by two factors: (a) prolonged exposure to increasingly high salinities for a period of months, permitted physiological acclimatization, or (b) the increasing salinities obliterated the non-regulating crabs, thus selecting for strong regulators which were the only crabs remaining to be observed. Of course, both factors may be involved. In order to test these two possibilities a group of *Hemigrapsus* collected from Los Penasquitos lagoon when the salinity was in excess of 160% sea water was back-acclimated by gradual steps to 100% sea water. After a period of about two months in 100% sea water the crabs were immersed directly into 150% sea water for a period of 72 hours. The blood was then sampled and analyzed for total cations. Of seven surviving specimens thus treated only one demonstrated significant hypo-osmotic regulation. The blood of this one crab was equivalent in concentration to 134% sea water which is not as strong regulation as the weakest regulator observed in a field situation where the lagoon water was 160% sea water (Fig. 1). Nevertheless, this is better regulation than has been reported for *Hemigrapsus* previous to the present investigation.

The results of this experiment are difficult to interpret because the loss of regulation may be a matter of physiological breakdown caused by the prolonged laboratory conditions. The experiment might be interpreted to mean that sudden transfer from normal to 150% sea water did not permit time for physiological acclimation which would occur in an isolated lagoon. Also, the fact that one specimen of this group was regulating in 150% sea water for a period of 72 hours, an ability not shown for *Hemigrapsus* either by Jones (1941) or Gross (1957a), suggests that some selection for hypo-regulation had taken place in the lagoon. Evidence was produced above that such selection had occurred in the case of *Pachygrapsus*. It seems reasonable that both selection and physiological acclimation were involved.

An attempt was made to acclimate *Hemigrapsus* gradually from 100% sea water to increasing salinities in order to demonstrate physiological acclimation, but the experiment failed because of a high mortality rate under the prolonged laboratory conditions.

Table I compares the blood ion concentrations of the two species of crab immersed in 100% sea water and 175% sea water. It should be pointed out that the

TABLE I

Ionic concentration in the blood of Hemigrapsus *and* Pachygrapsus

		100% sea water* (laboratory conditions)			175% sea water (lagoon field samples)		
		Mean	S.D.	No.	Mean	S.D.	No.
Na (meq/l.)	P	483	17.3	36	802	105	10
	H	452	10.3	6	726	39.3	10
	M	464			801		
K (meq/l.)	P	7.36	1.4	36	14.1	2.2	10
	H	8.13	1.1	6	12.5	1.9	10
	M	9.8			15.0		
Ca (meq/l.)	P	29.6	5.9	44	43.8	5.0	10
	H	37.7	5.3	5	51.0	10.3	10
	M	20.0			35.9		
Mg (meq/l.)	P	20.0	6.1	44	36.4	14.7	10
	H	70.0	13.3	5	135	31.4	10
	M	104			191		
Total (meq/l.)	P	540			896		
	H	568			925		
	M	598			1043		

P = *Pachygrapsus*
H = *Hemigrapsus*
M = Medium
* = Blood ion concentrations for *Pachygrapsus* in 100% sea water were reported previously (Gross, 1959).

values for crabs immersed in 175% sea water were determined from samples taken in the field. The values for normal sea water were for laboratory conditions for both species. However, the *Hemigrapsus* in this case were collected from Los Penasquitos lagoon returned to the laboratory and back-acclimated gradually to 100% sea water. Thus, the values for *Hemigrapsus* blood following immersion in normal sea water are subject to some question. Nevertheless, differences in the regulation of ions between *Pachygrapsus* and *Hemigrapsus* are salient in both salinities. In the first place, Na is regulated more strongly by *Hemigrapsus* than by *Pachygrapsus*. That is, the blood Na is significantly less concentrated in *Hemigrapsus* in both the normal and concentrated sea water than it is in *Pachygrapsus*, $P < 0.05$. On the other hand, blood Mg concentrations in *Hemigrapsus* are more than three times as high as they are in *Pachygrapsus* for both salinities. The regulation of K and Ca does not differ dramatically between the two species.

It is difficult to say on the basis of means that *Pachygrapsus* is the stronger hypo-osmotic regulator of the two species. Yet an examination of Figure 1 reveals that in the highest salinities where both species were studied, the strongest regulators, by far, were *Pachygrapsus* and the evidence is strong that *Pachygrapsus* survived *Hemigrapsus* at the extreme salinities. In view of these observations and the findings of earlier investigations (Jones, 1941; Gross, 1957a), it seems fair to consider *Pachygrapsus* the stronger regulating species in concentrated sea water. Yet it is suggested in Table I that the difference in osmo-regulatory ability is merely a difference in the ability to regulate Mg. That is, *Pachygrapsus* is a far stronger regulator of Mg than is *Hemigrapsus*.

Table II presents urine ion concentrations for the two crabs collected from 175% sea water. Since these samples were taken in the field, under conditions where contamination of urine samples with blood were more likely than under better controlled laboratory conditions, great precision cannot be placed on the concentration values. The mean urine Na for *Pachygrapsus* (572 meq/l.) is not significantly less than the mean urine Na for *Hemigrapsus* (687 meq/l.) although these concentrations would correlate well with the respective concentrations of Na in the blood of the two species. It is particularly interesting that the Mg concentration in the urine of the two species is about equal because as indicated above *Pachygrapsus* is a much stronger regulator of Mg than is *Hemigrapsus*. Values for urine Mg are smaller in *Pachygrapsus* than previously reported for lower salinities (Prosser *et al.*, 1955; Gross, 1959) which suggests that contamination with the blood had occurred. However, urine samples are more difficult to take from *Hemigrapsus* than *Pachygrapsus* and it is therefore likely that contamination of urine in *Hemigrapsus* also took place. It is concluded that while the values for urine Mg given in Table II are not precise, they strongly suggest that the antennary glands of both species are capable of concentrating Mg about equally.

Inspection of the total cation concentrations in urine and blood for both species (Table I and Table II) shows higher total ions in the urine than in the blood (meq/l.), but not knowing the anion constituents, this cannot be interpreted to mean that the urine is hypertonic to the blood. The blood and urine were compared by melting point determinations on six individual *Pachygrapsus* removed from the lagoon when the salinity was about 180% sea water. The average ratio, urine concentration/blood concentration, was 1.02 (range = 1.00 to 1.06). Thus, if the

TABLE II

Ionic concentration in field samples of urine taken from Hemigrapsus *and* Pachygrapsus *collected from 175% sea water*

		Mean	S.D.	No.
Na (meq/l.)	P	572	137	5
	H	687	84.8	8
K (meq/l.)	P	16.4	3.3	5
	H	12.0	1.9	8
Ca (meq/l.)	P	46.0	9.9	5
	H	56.9	14.9	8
Mg (meq/l.)	P	353	87.6	5
	H	364	195	8
Total (meq/l.)	P	987		
	H	1120		

P = *Pachygrapsus*
H = *Hemigrapsus*

urine is slightly more concentrated than the blood, it is not sufficiently so for the antennary glands to be effective as osmotic regulators.

Salinity preference was measured for *Pachygrapsus* collected from Los Penasquitos lagoon when the salinities were high at least six months after its isolation from the open sea. Nine crabs collected from the lagoon were individually placed in a salinity selectivity chamber for at least 36 hours where they could choose between 50%, 100%, 130% or lagoon water from which the particular crab had been collected (160%–180% sea water). A record was, therefore, obtained on the salinities visited and on the time spent in each. Adequate precautions were taken against choice for position rather than salinity. The procedure and apparatus are discussed by Gross (1957b). Thus, of the nine crabs, five preferred 100% sea water, two preferred 50% sea water, one preferred 130% sea water and only one demonstrated a preference for the salinity from which it had been collected. The mean time spent by all the crabs in the different salinities (% total time in the box) were: 50% sea water, 21.6; 100% sea water, 32.3; 130% sea water, 12.8 and lagoon water only 6.5% of the total time. It, therefore, seems likely that the preference for normal sea water previously described for *Pachygrapsus* persists even though the animal has been removed from normal sea water for months. It is even more apparent that it avoids the high salinities in which it had been living.

It has been reported by Gross and Marshall (1960) that the tissues of *Pachygrapsus* (as suggested by muscle) increase in water content when the animal is transferred from normal to dilute sea water and decrease in water content when the animal is transferred from normal to concentrated sea water, the resulting volume changes in the tissue taking place at the expense of the blood space. The above authors point out that such alterations in the anatomy of the blood space likely would affect the efficiency of the vascular system and consequently the adaptiveness of the animal. They suggested that volume control in the tissue might occur after a prolonged exposure to osmotic stress, thus permitting the vascular system to return to a normal anatomy.

Ten *Pachygrapsus* collected from Los Penasquitos lagoon on July 12, 1960, were returned to the laboratory in Riverside where they were immersed in lagoon water (183% sea water) for an additional 24 hours. Samples of muscle tissue were removed from the legs of each crab, blotted uniformly, weighed, and dried to constant weight in a drying oven at 95° C. The difference between dry weight and wet weight was considered to be the water content of the tissue. Thus, the mean water content of samples from each of ten crabs was 69.24% wet weight, S.D. = 1.46. Water content of muscle from *Pachygrapsus* immersed in normal sea water was reported as 75.00% wet weight, S.D. = 1.40; from 150% sea water, 71.70% wet weight, S.D. = 1.71 (Gross and Marshall, 1960). All the above values are significantly different; $P < 0.01$. It, therefore, becomes apparent that the muscle of *Pachygrapsus* does not return to normal volume when immersed in concentrated sea water for a period of months and this is probably true for the other formed tissues. Thus, if the volume of the blood space is returned to normal after prolonged exposure to osmotic stress it must be brought about by other means. Burger and Smythe (1953) suggest that the nephridial bladders and stomach of *Homarus* serve such a purpose.

DISCUSSION

The crabs *Pachygrapsus* and *Hemigrapsus* can be adapted to high salinities in two ways: (a) tolerance and (b) hypo-osmotic regulation. While the responses of *Pachygrapsus* to concentrated sea water in the field do not differ greatly from those reported for laboratory studies (Jones, 1941; Prosser *et al.,* 1955; Gross, 1957a), the responses of *Hemigrapsus* are quite different from laboratory findings and point to a case where short-term studies have led to an erroneous conclusion, namely that *Hemigrapsus* cannot hypo-regulate.

Pachygrapsus might be considered the stronger hypo-regulator of the two species and as indicated above, this is likely made possible by its stronger ability to regulate Mg. It is particularly interesting, however, that *Hemigrapsus* should be a weaker regulator of Mg than *Pachygrapsus* because it seems equally capable of concentrating this ion in its urine (Table II).

Now a crab which is regulating in a concentrated medium tends to lose water with the physical gradient and also by way of the urine it excretes. Yet as previously demonstrated (Gross, 1957a) the crab (at least *Pachygrapsus*) retains its normal body volume when immersed in concentrated sea water. This means, of course, that an uptake of water must balance the loss. A highly permeable animal, losing relatively large volumes of water to the external medium by diffusion, could thus exclude only a small fraction of its water intake in the form of urine. Also, if water loss were compensated for by drinking, then a highly permeable animal, experiencing a large water loss, would drink relatively large volumes of the concentrated medium which would mean a large intake of Mg. There is evidence that the exoskeleton of *Hemigrapsus* is more permeable to water than that of *Pachygrapsus* (Gross, 1957a). It may be that *Pachygrapsus* because of its lower permeability can exclude a greater fraction of its water intake in the form of urine, than can *Hemigrapsus* which in turn would mean a greater exclusion of Mg. It might also be that *Pachygrapsus* having a smaller water turn-over does not drink as much as does *Hemigrapsus* and consequently takes in less of the Mg-rich water.

It was indicated above that the crabs (particularly *Hemigrapsus*) of Los Penasquitos lagoon migrated out of the depths to the shores' edge when the salinities became high, yet it cannot be concluded that salinity *per se* caused this movement for there were other physical and biological factors involved. For example, the oxygen tensions in the depths of the lagoon were extremely low in the summer months and by the end of August, 1959, were nil (Carpelan, 1961). It is believed that the lack of oxygen was the most critical stress forcing the migration. There was some rotenone poisoning in limited areas of the lagoon on several occasions during the summer of 1959 (Carpelan, 1961). However, this is not believed to have influenced the migration because the poison is a highly unstable substance which could be effective only for a short period in the limited areas where used. Besides, rotenone was used in early June and July, yet *Hemigrapsus* did not move into the shallows until August. Surface temperatures for several locations in the lagoon remained between 25° and 29° C. during July, August and September of 1959 (Carpelan, 1961). Thus, it does not seem that temperature could be critical in forcing the shoreward migration.

Los Penasquitos lagoon presents the physical stress which would tend to select for hypo-osmotic regulation, that is, high salinities. The lagoon also becomes stagnant, and in acquiring anaerobic conditions presents a stress which could force animals capable of movement toward the shore and out of the water. Thus, a selective pressure favoring aerial respiration is presented by the lagoon. While there are no available records of the salinities of this lagoon during the rainy season of a wet year, Carpelan (1961) has shown significant decreases in salinity following the light rains of 1959 and it is likely that run-off during heavy rains could dilute the lagoon water considerably.

If dilute as well as hypersaline conditions in coastal lagoons of the past were common, then the correlation between osmotic regulation and terrestrial life among crabs can be explained as a reflection of the history of the animals rather than necessarily a physiological adaptation to habitats in which the animals are typically found today. The adaptive significance of osmotic regulation in semi-terrestrial and terrestrial crabs was questioned by Gross (1955).

It is suggested, therefore, that coastal lagoons might have afforded ideal conditions for the evolution of terrestrial crabs. That is, selective pressures are available in these bodies of water favoring: (1) hyper-osmotic regulation, (2) hypo-osmotic regulation, and (3) aerial respiration, three physiological conditions common to crabs showing degrees of the terrestrial habit.

It probably would have been necessary for the crabs to be trapped as were the Los Penasquitos populations so that obligatory selection would have been imposed upon them. Also it probably would have been necessary that access to the open sea and normal salinities be seasonally available. Inasmuch as ovigerous crabs were not found in Los Penasquitos lagoon when the salinities were high, it is unlikely that reproduction occurred in the lagoon during the stressed conditions. However, this does not mean that such a population would be non-breeding, for if the isolated body of water were opened to the sea periodically, then normal salinities would become available which would permit reproduction and the escape of free-swimming larvae to the open sea. It may be that the retention of a preference for normal sea water in *Pachygrapsus* after months in hypersalinities is an adaptation which assures reproduction in normal salinities. The periodic availability of normal sea water might explain why the land crabs have not evolved reproductive mechanisms for terrestrial life.

It does not follow that lagoons would have had to dominate the coastal areas where land crabs evolved, although lagoons which are opened intermittently to the sea during high equinoctical tides and rainy seasons are common today on the coast of South Africa, Brazil, Australia and south Texas (Ladd *et al.,* 1957). Neither would it be necessary for the majority of a particular species to inhabit the lagoons. All that would be required would be large absolute populations within the lagoons where they would be rigorously selected for the three physiological features which would have to have hereditary components. It also would be required that the three physiological factors would not be selected against in normal sea water. Thus, adults which were severely selected in an isolated lagoon for the ability to regulate osmotically and to respire in the air would periodically reproduce. Their young would then escape to the open sea and in effect increase the gene frequency for the characters in question within the general population. Given sufficient time, the characters could be common to the species. Of course, once the first step was made toward terrestrial life selective pressures might favor this feature in situations outside of the lagoon.

Edney (1960) emphasizes that the evolutionary route toward land directly across the littoral zone from the sea is more difficult than by way of estuaries and swamps because the problems of terrestrial adaptation are imposed at once. Gislén (1947) considers estuarine mangrove swamps as most likely pathways toward terrestrial life. Hedgepeth (1957) discusses the open marine beach as a direct route for landward migration. Neither of these possibilities, however, explains the common occurrence of both hyper- and hypo-osmotic regulation among terrestrial crabs.

On the other hand, conditions in coastal lagoons such as described above could not only provide the selective pressures favoring both types of osmo-regulation, but also could provide the conditions whereby the initial step from sea to land could be evolved in a gradual, but obligatory manner.

The direct invasion of land across a marine beach would require an initial step where the stresses of aerial life would be imposed suddenly. It would seem that the disadvantage of such a sudden step would outweigh the advantages of becoming land-borne. Perhaps crabs which appear to have become land-borne directly across the littoral zone (*e.g. Ocypode*) have in their history experienced selective pressures favoring such a move in ecological situations (lagoons of varying salinity) which differ from their present typical habitats.

Ferronnière (1901) was one of the earlier workers to point out that animals capable of tolerating low salinities commonly are also capable of tolerating high salinities. He also observed that it is common for species of the supra-littoral zone to inhabit brackish as well as hypersaline waters.

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SUMMARY

1. The crabs *Pachygrapsus crassipes* and *Hemigrapsus oregonsis* were found thriving in an hypersaline lagoon which had been isolated from the sea for months and had attained salinities in excess of 175% of normal.

2. Contrary to previous reports *Hemigrapsus* was found to be a strong hypo-osmotic regulator in concentrations as high as 175% sea water. The discrepancy between previous laboratory findings and the field results of the present investigation are attributed to the prolonged acclimatization period in lagoon water of gradually increasing salinity and to severe selection permitting only hypo-regulators to be sampled.

3. No living *Hemigrapsus* were found in the lagoon when the salinities had exceeded 180% sea water.

4. *Pachygrapsus,* as was expected from previous studies, was regulating osmotically in the lagoon when the concentrations were above 185% sea water.

5. Both *Pachygrapsus* and *Hemigrapsus* can tolerate blood concentrations in excess of 160% sea water and remain active.

6. *Pachygrapsus* is a stronger hypo-osmotic regulator than *Hemigrapsus.* Since it maintains its blood Mg considerably lower than does *Hemigrapsus* in concentrated sea water, the stronger ability to hypo-regulate may be made possible by the greater capacity on the part of *Pachygrapsus* to excrete Mg.

7. The urine Mg for *Hemigrapsus* is about equal in concentration to that of *Pachygrapsus* when the crabs are immersed in 175% sea water, even though the blood Mg concentration is more than three times higher than that of *Pachygrapsus.*

8. *Hemigrapsus* is a stronger regulator of Na in hypersaline water than is *Pachygrapsus.*

9. The antennary glands in both *Pachygrapsus* and *Hemigrapsus* are ineffective as organs of hypo-osmotic regulation.

10. When *Pachygrapsus* is transferred from normal to concentrated sea water the volume of its muscle tissue, as indicated by water content, becomes reduced. This reduction in volume persists in hypersaline waters even after months of immersion. Thus, there is no evidence of volume control for muscle tissue.

11. After months of isolation in a hypersaline lagoon *Pachygrapsus* shows a preference for normal sea water when offered a choice of salinities and will avoid the hypersaline water from which it has been captured.

12. Evidence is produced showing that coastal lagoons present selective pressures favoring: (1) hyper-osmotic regulation, (2) hypo-osmotic regulation, and (3) aerial respiration, three physiological characteristics common to terrestrial and semi-terrestrial crabs. Coastal lagoons are suggested as ideal sites for the evolution of land crabs.

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